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DOI:

[10.1016/j.physbeh.2018.02.013](https://doi.org/10.1016/j.physbeh.2018.02.013)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Heaney, J, Killer, S, Svendsen, IS, Gleeson, M & Campbell, J 2018, 'Intensified training increases salivary free light chains in trained cyclists: indication that training volume increases oral inflammation', *Physiology and Behavior*, vol. 188, pp. 181-187. <https://doi.org/10.1016/j.physbeh.2018.02.013>

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Checked 7.2.2018

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**Intensified training increases salivary free light chains in trained cyclists: indication that
training volume increases oral inflammation**

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Abstract

Periods of short-term intensified training (IT) are often used by athletes during training cycles over the season and undergoing phases of increased physical stress may impact upon the immune system. This study investigated the effects of a period of IT on free light chains (FLCs) in saliva - an emerging immune biomarker of oral inflammation - and matched serum samples in well-trained athletes. It also examined if IT influences basal FLC levels and FLC flux during acute exercise. Highly trained male cyclists ($n = 10$) underwent a 9-day period of IT; before and after IT participants performed a 1 h time trial (TT) on a cycle ergometer, with blood and saliva samples collected pre- and post-exercise. FLCs were assessed in serum and saliva, and IgG, IgA, IgM and creatinine were also measured in serum. Weekly training volume increased by 143% (95% CI 114–172%), $p < 0.001$, during IT compared with pre-trial baseline training. Following IT, the cyclists demonstrated higher salivary FLC levels. Both salivary lambda FLC concentrations ($p < 0.05$, $\eta^2 = .384$) and secretion rates, and kappa FLC concentrations and secretion rates increased after IT. Salivary FLCs concentration and secretion rates decreased in response to the TT following IT ($p < 0.05$, $\eta^2 = .387-.428$), but not in response to the TT prior to IT. No significant effects of IT on serum FLCs were observed. There were no significant changes in serum FLCs in response to the TT, before or after the IT period, nor did IT impact upon other serological responses to the TT. In conclusion, IT increased basal salivary FLC parameters and amplified decreases in salivary FLCs in response to acute exercise. Increases in salivary FLC concentration likely reflects alterations to oral inflammation during times of heavy training, and we show for the first time that FLCs may have utility as a marker of exercise stress and oral health status.

Key words: free light chains, saliva, exercise, training, athletes, inflammation

1. Introduction

Periods of short-term intensified training (IT) or over-reaching are often used by athletes during training cycles over the course of a season to elicit performance gains. Whilst IT may improve an athlete's physiological and performance capacities, it is thought that there may be detrimental consequences to host health. Perturbations to several aspects of cellular and mucosal immunity have been reported following short-term (1–3 weeks) IT in trained athletes [1, 2]. It is speculated that immune changes may compromise host defence and lead to a heightened risk of upper respiratory tract infections (URTI). Salivary IgA (sIgA) has been associated with the incidence of URTI in athletes and progressive decreases in sIgA have been observed during periods of IT [2]. However, strong evidence of the link between exercise, oral immunity and susceptibility to infections is still lacking. Surprisingly, an aspect of immune competency that has received little attention are immune biomarkers of oral inflammation. Indeed, athletes have been shown to have particularly poor oral health [3]. Athletes attending the Dental Clinic of the 2012 Olympic Games presented with high rates of periodontal disease [4]. Poor oral health has been negatively associated with self-reported performance leading to recommendations for greater consideration of this factor for optimal performance for elite athletes [3-5]. However, to what extent short-term periods of intensive training affect oral inflammation in athletes is not well characterised. Identification of biomarkers to monitor 'exercise stress' may be of utility in high-performance sport for detecting those at risk of performance deficiency or adverse well-being and health effects.

Immunoglobulin free light chains (FLCs) have recently been quantified for the first time in human saliva and are considered an emerging biomarker of oral inflammation [6]. Light chains are produced by plasma cells in excess of heavy chains, meaning that for every intact immunoglobulin – comprised of two identical heavy chains and two identical light chains – that is produced, there is abundant light chain (FLC) secreted by plasma cells into the systemic circulation [7]. Serum FLCs and the ratio of kappa:lambda light chains (K:λ ratio) are central in the diagnosis, prognosis and monitoring of plasma cell malignancies, such as multiple myeloma, where serum FLC analysis is recommended internationally for measurement of involved FLC from monoclonal plasma cells [8-11]. FLCs are also dysregulated in a range of non-malignant disorders. For example, elevated

polyclonal FLCs have been observed in a range of conditions including: rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, heart failure, diabetes, renal disease, asthma, chronic obstructive pulmonary disease, inflammatory bowel disease and HIV infection [12-14]. In these conditions FLCs are useful as a marker of disease activity and for predicting disease progression. In the general population, combined levels of kappa and lambda FLCs (FLC sum) have been shown to be prognostic for all-cause mortality, whereby FLC sum negatively predicted survival in a large cohort of individuals aged ≥ 50 years without plasma cell disorders [15]. Using a method of sensitive quantitation for FLCs in saliva, we found that substantially higher levels of salivary FLCs are present in elderly individuals compared with younger adults, indicative of poorer overall oral health and a greater degree of local inflammation and immune activation in this age group [6].

To date, the impact of short-term changes in exercise training load on FLC levels in saliva or serum has yet to be investigated. There is evidence to suggest that FLCs in both saliva and blood could be affected by changes to training loads, whereby increases in training load may negatively impact upon oral health or disrupt local immune and inflammatory processes that may translate into perturbations in salivary FLCs. Decreases in B cells have been shown in athletes after an intense training camp [16], which in turn could result in reductions in FLC levels. Furthermore, undergoing a period of increased physical stress could impact upon how the immune system responds to an acute exercise challenge. The effects of acute exercise on salivary FLC concentration and secretion rates appear dependent on age; significant reductions in salivary FLCs have been observed post-exercise in elderly adults, where only modest reductions occurred in young adults [6]. Kappa FLCs were found to be only marginally elevated in runners following a marathon compared with pre-exercise levels, thought to be indicative of perturbations to renal function proportional to exercise intensity [17]. Consistent with this, other studies of lower exercise intensity have found no changes in serum FLCs in response to exercise [6, 18]. One study investigated the effects of IT on immune responses to acute exercise and generally found no changes based on increased training load [19]. However, to our knowledge very few other studies have since investigated this and the effects of IT on FLC responses to acute exercise have not

been explored. The primary aim of the present study was to assess the effects of a period of IT on FLCs in saliva and serum in well-trained athletes, and secondly, to examine if IT influences FLC responses to acute exercise.

2. Materials and Methods

2.1. Participants

Highly trained male cyclists ($n = 10$) with a competitive cycling background of at least 3 years, cycling ≥ 3 times per week for a minimum of 2 h per day, participate in this study. Participants had a mean \pm SD age of 23.6 ± 4.0 years; BMI of 21.6 ± 1.5 kg/m²; VO_{2max} of 72.7 ± 4.8 mL/kg/min. Inclusion criteria stipulated that participants were not suffering from any acute illness in the two weeks prior to, or during the study and they had no chronic illness, history of chronic illness, nor were taking any medications. Informed consent was obtained from all individual participants included in the study.

2.2. Study design

Participants underwent pre-study testing to confirm suitability to take part in the study. After enrolment into the study, participants' exercise performance was assessed before and after a period of IT [20]. Participants performed a 1 h time trial on a cycling ergometer, with blood and saliva samples collected pre- and post-exercise; then underwent a 9-day period of IT cycling; participants then performed another 1 h TT with blood and saliva samples collected pre- and post-exercise.

2.3. Pre-study testing and familiarisation

Participants visited the laboratory for pre-trial exercise testing to confirm individuals were highly trained (VO_{2max} of ≥ 65 mL/kg/min) and therefore suitable to be included in the study. During this visit, participants performed an incremental cycle test to exhaustion on an electronically braked ergometer (Lode Excalibur Sport, Groningen, Netherlands) to assess VO_{2max} . Expiratory gases were collected continually throughout the test and breath-by-breath analysis was performed

automatically with a Moxus metabolic systems analyser (AEI Technologies Inc., Naperville, IL, USA). Heart rate (HR) was recorded continually using short range telemetry (Polar RS800CX, Polar, Kempele, Finland) and ratings of perceived exertion were recorded from participants during the final minute of each stage. Suitable participants then recorded a 2-week training diary, were provided with a HR monitor (Suunto ANT, Amer Sports Corporation, Finland) and a SRM power meter (SRM Shimano DA7900 PowerMeter) to monitor their training. Participants were also requested to train according to their normal programmes during this phase. Following the pre-trial phase, participants began 9 days of IT.

2.4. Intensified Training

Individual IT programmes were based on participants' pre-trial training diaries and discussed with each individual athlete. Training volume and intensity defined by HR (time spent in the highest 3 HR zones) was increased 2.5-fold from baseline. HR zones were calculated from maximum HR achieved during the pre-study VO_{2max} test. Five zones were devised, and expressed as a percentage of maximum HR; Zone 1: <69 %, Zone 2: 69 %–81 %, Zone 3: 82 %–87 %, Zone 4: 88% –94 %, Zone 5: >94 % [21]. Power zones were also applied to interval sessions: these were calculated as a percentage of peak power (W) attained in each participant's VO_{2max} test and used as a guideline during training sessions, alongside HR. We expected the IT would reduce a participant's ability to achieve the same distribution of HR zones; therefore, power zones were put in place as an additional measure to encourage participants to achieve the highest workload possible. Participants uploaded data files (power and HR) after each training session via secure online training software (Training Peaks, Boulder, CO, USA.). This process enabled the researchers to monitor sessions not performed in the laboratory.

2.5. Time Trials

Participants arrived at the laboratory between 06:30-08:30 following an overnight fast of ≥ 8 h. Blood samples were collected from an antecubital vein from participants in a seated position. Unstimulated saliva samples were collected during a 3-min collection period from participants in the seated position. Following a 10-min warm-up at a self-selected intensity, participants

performed an all-out 1-h TT on their own bicycle on a Turbo Trainer (CycleOps Flow, Madison, WI, USA) in a quiet laboratory environment at $20 \pm 1^{\circ}\text{C}$. HR was recorded continually throughout each TT with short range telemetry (Suunto, Vantaa, Finland). Participants were blinded from their power, cadence and HR during the TT and only provided with a stop clock. Participants were permitted to drink water *ad libitum* during TT, except for the final 10 min. Post-TT blood and saliva samples were collected within 5 min of exercise cessation.

2.6. Blood and saliva samples

Blood was collected by venepuncture into plain vacutainer tubes (BD Vacutainer, Plymouth, UK). Whole blood samples were allowed to clot at room temperature for 20 min and then centrifuged at 5,000 *g* for 10 min at 4°C ; separated serum was immediately stored at -20°C until analysis. Unstimulated saliva samples were collected with participants in the seated position, leaning forward with their head tilted forward. Participants were asked to swallow to empty their mouth of any residual saliva before the timed sample collection began. Saliva collections lasted 3 min, during which time participants were requested to minimise orofacial movement and passively dribble into a pre-weighed vial. Samples were weighed to estimate saliva volume assuming a saliva density of 1.00 g/mL; saliva flow rate (mL/min) was determined by dividing the volume of saliva by the collection time. Samples were transferred to Eppendorf tubes and centrifuged at 10,000 *g* for 2 min to separate cells and insoluble matter. The supernatant was then removed and stored at -20°C until assay.

2.7. Salivary free light chain assays

Salivary kappa and lambda FLCs were quantified using highly sensitive sandwich ELISAs developed by the Clinical Immunology Service at the University of Birmingham. These assays use monoclonal antibodies (mAbs) that have been extensively characterised and validated previously [22]. The microtitre wells were coated with mAbs (Abingdon Health, York, UK) that specifically target either human kappa or lambda FLC and thus do not bind light chain in whole immunoglobulin. After a blocking period of 1 h to prevent non-specific binding, standards, controls and saliva samples were added to the plate in duplicate. After 1 h incubation, plates were washed

to remove any unbound sample. Kappa or lambda detection antibody labelled with horseradish peroxidase was then added to the plate and left to incubate for 1 h. Detection antibodies were mAbs specific for kappa or lambda light chains either free or bound, that recognise an epitope distinct from the one used to capture the FLCs from saliva (Abingdon Health, York, UK). Plates were washed again and substrate solution was added; after 10 min incubation, the reaction was stopped and the optical density was measured at 450 nm. The intra-assay CVs were < 10% and inter-assay CVs were < 15%. This assay has been described in more detail previously [6].

2.8. Serum free light chain analysis and other serological parameters

Serum kappa and lambda FLCs were quantified using a multi-plex bead-based assay using a Luminex platform (Bio-plex systems, BioRad Laboratories, California, USA). This assay was developed by the Clinical Immunology Service at the University of Birmingham and uses mAbs specific for either kappa or lambda FLC (Abingdon Health Ltd, Oxford, UK) in a competitive inhibition format. Full details for this assay have been described previously [22]. Serum creatinine, IgG, IgA and IgM were measured on a Roche Hitachi Modular. To minimize variation, all Roche Hitachi Modular analyses were performed on a single day using the same calibration set-up, batches, and user. Using serum creatinine, renal function was assessed by calculating estimated creatinine clearance rates (eCrCl) and estimated glomerular filtration rate (eGFR) using the Cockcroft-Gault ($[(140 - \text{age}) \times \text{mass} \times 1.23 / \text{serum creatinine}]$) and Modification of Diet in Renal Disease ($186 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ if female) formulae, respectively.

2.9. Free light chain parameters

A range of FLC parameter outputs were analysed as part of this study. In addition to absolute concentrations of kappa and lambda FLC, the ratio of kappa to lambda FLC (K: λ ratio) and the difference between kappa and lambda FLC (FLC difference) were examined. The K: λ ratio and FLC difference are conventionally used in conditions involving perturbed levels of FLC, such as plasma cell disorders [23, 24]. The sum of kappa and lambda FLCs (FLC sum) was also investigated as this measure has been employed in the general population as a global indicator of health status [15]. In addition to protein concentrations, saliva secretion rates of immunoglobulins

are typically reported to reflect the total availability of protein at the oral surface and control for hydration status [25]. Secretion rates of FLCs ($\mu\text{g}/\text{min}$) were calculated as saliva flow rate \times kappa/lambda concentration. The other parameters (sum, difference and ratio) were also expressed in this way to control for any impact of saliva flow rate upon these variables.

2.10. Data analyses

Analyses were undertaken using IBM SPSS version 21. One-way repeated measures ANOVA were used to assess differences in I) training volume and intensity before IT and during IT; II) resting FLCs in serum and saliva and serological parameters in serum before and after IT; III) FLCs in serum and saliva and serological parameters between pre- and post-exercise, prior to and after IT. If data were not normally distributed, statistical analysis was performed on the logarithmic transformation of the data. Slight variations in degrees of freedom reflect insufficient serum for analysis of all parameters. Greenhouse-Geisser corrected F values are reported for repeated measures analyses and partial η^2 , a measure of effect size, with guidelines of small (> 0.01), medium (> 0.06) and large (> 0.14), is reported throughout.

3. Results

3.1. Intensified training

Weekly training volume increased significantly during IT compared with pre-trial baseline training (143% increase [95% CI 114 to 172%]: 9.4 ± 2.2 to 22.4 ± 3.9 h/week; $p < 0.001$). Total time spent training during the 9 days of IT was 28.8 ± 5.0 h. Weekly training intensity increased significantly during IT compared with pre-trial baseline training (166% increase [95% CI 43 to 290%]: 2.8 ± 1.3 to 6.8 ± 4.4 h/week spent training above 82% HRmax; $p < 0.05$). Total time spent training above 82% HRmax during IT was 8.7 ± 5.7 h.

3.2. The effects of intensified training on resting salivary free light chains

Following the period of IT, higher FLC concentrations were observed in the cyclists. This was driven by a significantly higher lambda FLC concentration pre-exercise, compared with pre-exercise at baseline: $F(1,9) = 5.61$ $p = .042$, $\eta^2 = .384$ (Figure 1). Also indicated in this Figure are higher pre-exercise kappa FLCs after IT, although this increase did not reach statistical significance. There was also a trend for resting saliva kappa and lambda FLC secretion rates to increase following the IT period (Figure 2). The corresponding percentage changes in salivary FLC concentrations and secretion rates after IT are illustrated in Figure 3. In general, other resting saliva parameters (sum, difference and ratio) for both concentrations and secretions, increased following IT; descriptive statistics before and after IT are shown in Table 1.

Table 1 Mean \pm standard deviation salivary and serum free light chain (FLC) responses in young male trained cyclists, at rest (pre-exercise) and in response to exercise, before (baseline) and after a period of intensified training

	Baseline		Post-intensified training	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Saliva flow rate (mL/min)	0.62 \pm 0.34	0.52 \pm 0.18	0.63 \pm 0.37	0.53 \pm 0.21
Saliva concentrations (mg/L)				
FLC sum	2.50 \pm 2.17	1.37 \pm 0.91	3.59 \pm 2.79	1.91 \pm 1.19*
FLC difference	0.68 \pm 0.96	0.14 \pm 0.59	0.65 \pm 1.46	0.65 \pm 0.92
K: λ ratio	2.38 \pm 1.22	1.88 \pm 1.94	2.65 \pm 1.88	2.45 \pm 1.83
Saliva parameters controlling for flow rate (μ g/min)				
FLC sum	1.17 \pm 0.69	0.65 \pm 0.40	1.74 \pm 1.06	0.94 \pm 0.56*
FLC difference	0.38 \pm 0.44	0.08 \pm 0.22	0.47 \pm 0.80	0.33 \pm 0.44
K: λ ratio	1.47 \pm 1.03	0.91 \pm 0.70	1.77 \pm 1.62	1.30 \pm 0.95

* Significant decrease compared to pre-exercise, post-intensified training, $p < 0.05$

3.3. The effects of intensified training on resting serum parameters: free light chains, immunoglobulins and creatinine

There was no significant effect of IT on resting pre-exercise serum FLC levels: small mean changes of -2.8% (95% CI -11.7 to 6.1%) and -5.8% (95% CI -13.4 to 1.7%) were observed for kappa and lambda FLCs, respectively. These percentage changes were much smaller relative to those observed in saliva and showed a decrease rather than an increase (concentrations of serum FLCs are contrasted with saliva in Figure 1). Values for other serum FLC parameters (sum, difference and ratio) for are reported in Table 2 and did not change significantly following IT. In general, serum immunoglobulins decreased after IT (Table 2, pre-exercise values). However, this decrease was only significant for IgA, $F(1,9) = 6.14$ $p = .035$, $\eta^2 = .406$. The mean percentage changes in immunoglobulins were equivalent to -4.1% for IgG (95% CI -14.2 to 6%), -6.0% for IgA (95% CI -11.8 to -0.1%) and -2.6% for IgM (95% CI -33.2 to 28%). There were no changes in serum creatinine levels following IT, eCrCl or eGFR (mean values shown in Table 2).

3.4. Salivary free light chain responses to a 1 h all-out cycling time trial, before and after intensified training

At baseline, prior to the IT period, mean saliva variables generally registered lower post-exercise values compared with pre-exercise. However, these did not translate into significant changes in salivary FLC concentrations, FLC secretion rates (Figures 1 and 2, baseline exercise bout) or any other saliva variables in response to exercise (Table 1, baseline). In contrast to baseline, the exercise bout after IT resulted in significant decreases in salivary FLCs. As presented in Figures 1 and 2, kappa concentration ($F(1,9) = 5.68$ $p = .041$, $\eta^2 = .387$), kappa secretion ($F(1,9) = 6.65$ $p = .030$, $\eta^2 = .425$) and lambda secretion ($F(1,9) = 6.74$ $p = .029$, $\eta^2 = .428$) were significantly reduced in response to the second TT. Further, FLC sum ($F(1,9) = 7.03$ $p = .026$, $\eta^2 = .438$), and FLC sum adjusted for flow rate ($F(1,9) = 8.86$ $p = .016$, $\eta^2 = .496$) also significantly decreased (Table 1). No other saliva parameters changed significantly in response to exercise.

Table 2 Mean \pm standard deviation serum free light chains, immunoglobulin, creatinine, estimated creatinine clearance rate (eCrCl) and estimated glomerular filtration rate (eGFR) responses in young male trained cyclists, at rest (pre-exercise) and in response to exercise, before (baseline) and after a period of intensified training

	Baseline		Post-intensified training	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Serum FLCs (mg/L)				
FLC sum	23.23 \pm 2.85	23.44 \pm 4.18	22.26 \pm 4.35	23.89 \pm 3.90
FLC difference	-2.89 \pm 2.23	-2.67 \pm 1.82	-2.45 \pm 1.77	-3.23 \pm 2.14
K: λ ratio	0.79 \pm 0.15	0.80 \pm 0.12	0.81 \pm 0.12	0.77 \pm 0.14
Immunoglobulins (g/L)				
IgG	9.40 \pm 1.54	9.90 \pm 1.80*	8.96 \pm 1.74	9.54 \pm 1.75
IgA	1.86 \pm 0.51	1.94 \pm 0.54	1.74 \pm 0.46 [†]	1.89 \pm 0.39
IgM	1.14 \pm 0.38	1.19 \pm 0.40	1.03 \pm 0.33	1.06 \pm 0.32
Creatinine (umol/L)	97.00 \pm 9.72	105.50 \pm 14.12	98.20 \pm 11.71	108.33 \pm 17.76
eCrCl (mL/min/1.73m ²)	102.01 \pm 15.59	94.63 \pm 17.83*	101.03 \pm 15.94	92.55 \pm 17.42*
eGFR (mL/min/1.73m ²)	89.21 \pm 10.24	81.67 \pm 12.40*	88.45 \pm 12.57	79.62 \pm 15.00*

* Significant compared to pre-exercise, $p < 0.05$

† Significant compared to pre-exercise at baseline, $p < 0.05$

3.5. Serological responses to a 1 h all-out cycling time trial, before and after intensified training

As shown in Figure 1, there were no significant changes in serum FLCs in response to the TT, before or after the IT period. There were also no significant changes in FLC sum, difference or ratio parameters in response to either bout of exercise (Table 2). In comparison to saliva concentrations the changes in serum FLCs were small; mean percentage changes in responses to exercise were up to -120% for saliva and < 12% for serum. As shown in Table 2, serum immunoglobulins increased between pre- and post-TT both at baseline and after IT. With the exception of IgG at baseline ($F(1,9) = 9.15$ $p = .014$, $\eta^2 = .504$), these immunoglobulin increases in response to exercise were not significant. Similarly, serum creatinine increased in response to the TT, both before and after IT, but not significantly. However, the increases in serum creatinine were

sufficient enough to significantly decrease eCrCl and eGFR post-exercise both before ($F(1,9) = 27.81$ $p = 0.001$, $\eta^2 = .755$ and $F(1,9) = 28.22$ $p < 0.001$, $\eta^2 = .758$, respectively) and after IT ($F(1,8) = 12.54$ $p = 0.008$, $\eta^2 = .610$ and $F(1,8) = 11.57$ $p = 0.010$, $\eta^2 = .589$, respectively).

4. Discussion

The results of the present study demonstrated that a period of 9-days of IT increased the concentration of resting salivary FLCs in trained cyclists. Significantly higher lambda FLC concentrations were found and elevations in other parameters, lambda concentration, kappa and lambda secretion rates, and FLC sum, difference and ratio (with and without controlling for flow rate) were also observed. Although all parameters showed the same pattern of elevation, changes in concentrations were not consistent, statistical significance was not consistent, most likely reflecting the modest sample size of the study; it is likely that more uniform changes would have been observed for all of these parameters with a larger sample size, though further studies are now warranted to confirm this. Minimal changes were observed in serum FLCs, suggesting the effects of IT on FLCs are isolated to the oral mucosa rather than systemic bloodstream.

The exact route of FLC entry into saliva has yet to be confirmed but is likely a combination of direct gingival passive transfer from blood and local production from plasma cells. Thus, IT may increase localised production of FLCs or alternatively may promote transport of FLCs into the saliva, possibly due to changes in oral tissues. Indeed, FLCs may enter the saliva via gingival crevicular fluid (GCF), an oral fluid that consists of serum transudate and tissue exudates and has been shown to include immunoglobulins [26-28]. The volume and flow of GCF increases during inflammation [29], mainly due to increased vascular permeability [30], and is an indicator of gingivitis and periodontitis [27]. Further, increases in inflammatory markers in GCF with gingivitis have been seen previously [31]. Due to the role of FLCs in immune and inflammatory processes, the observed increases in saliva may represent greater oral inflammation and immune activation, and thus potentially increased FLC influx into the saliva through GCF, following increased training load. This could be due to deterioration in oral health during periods of IT. Athletes have been shown in general to have poor oral health and Olympic athletes have been found to have a high

incidence of periodontal disease, with the majority (75%) of individuals presenting with gingivitis and 15% periodontitis [3, 4]; these rates are above what has been reported in the general population in the US and UK [32, 33]. The physical stress of increasing training load may directly affect oral health or indirectly through changes in oral hygiene practices, sleep or nutritional factors. However, it should be remembered that oral health was not specifically measured in this study and the cause of increase FLCs cannot be confirmed. Associations between FLCs and oral health should be assessed in future studies. Further, the relationship between FLCs in GCF and whole saliva, with and without inflammation, needs to be assessed to understand the mechanism behind these findings and their clinical consequences.

Previous studies have shown alterations in immune parameters following short-term IT, and the current study extends these observations to FLCs. Immune parameters have been shown to decrease with IT accompanied by a shift towards an anti-inflammatory immune profile, which has been implicated in increased risk of URTIs in athletes [19, 34, 35]. The increase in FLCs implies a local pro-inflammatory effect, suggesting that the oral environment may respond differently to IT. Another oral measure, SIgA, has been shown to decrease with IT and this has been associated with increased incidence of URTIs [19, 36, 37]. The balance between FLCs and SIgA may be indicative of oral inflammation vs mucosal defence, and would be interesting to investigate in relation to IT and infection incidence. Further, oral health alongside immunity should be examined further in athletes, particularly during periods of IT: if oral health compromises local protection, this may be a mechanism/mediator of increased infection risk in athletes.

The present study followed athletes over a relatively short time period (< 2 weeks) and it would be valuable to extend monitoring throughout cycles of training to see assess fluctuations in FLCs. On return to normal training FLCs may return to baseline values, alternatively, continuation of IT may exacerbate elevations in FLCs. When IT is sustained with an imbalance between training and recovery, non-functional over-reaching may occur, and, if prolonged further, athletes could develop overtraining syndrome, characterised by long-term impaired performance [1]. Currently there is a lack of biomarkers able to identify transitions between IT-non-functional over-reaching and

overtraining and those athletes at increased risk. Low-grade systemic inflammation and immune dysregulation have been implicated as mediators in the development of overtraining syndrome [38, 39]. However, immune parameters such as leukocyte counts, neutrophil function and SIgA levels are not strong predictors of overtraining [2]. Changes in FLCs with IT were proportionately greater in magnitude than those seen with acute exercise, one of the criteria for being able to monitor training states [1], although individual variability in responses was seen in this relatively small number of athletes. For potential markers of training stress, it has been advised that individual profiles are obtained and samples are compared back to healthy baseline samples [2]. Thus in a future study, FLCs would need to be measured repeatedly in individuals over the course of the season to determine how FLCs respond to dynamics in training load to assess: firstly, if they are capable of sensitively detecting changes in training states, and secondly, if levels change prior to underperformance/ physiological or psychological symptoms as a potential prognostic marker of athletes at risk of entering states of non-functional overreaching or overtraining.

The baseline TT did not result in any significant changes in saliva FLCs, although parameters were typically reduced post-exercise. In contrast, after IT significant decreases in salivary FLCs were seen in response to the TT for concentrations, lambda secretion rate and FLC sum. Interestingly, significant decreases in salivary FLCs have only been seen in older adults and not young adults previously [6], suggesting IT may make salivary FLCs more susceptible to the effects of acute exercise. Changes in response to exercise are not explained by flow rate and may be due to changes in FLC transport into saliva with exercise; why this only occurs in response to exercise following IT is unclear. If FLC transport is affected by blood flow, independently of saliva volume, then one possibility is that following IT a greater re-direction of circulation to the working muscles is required to sustain exercise performance. Further, it remains to be seen how long salivary FLCs are reduced following exercise and if these changes have any implications for the health of athletes.

In general, the serum parameters measured in this study did not significantly change following IT. Whole immunoglobulins decreased but reductions were generally small (< 6%). Long-term intensive training has been associated with lower serum immunoglobulins in competitive athletes

compared to a moderately active control group [40]. Despite reductions in immunoglobulins following the short-term bout of IT, in the present study all immunoglobulin levels remained within the normal range and changes were not indicative of immunosuppression. Serum creatinine levels, and thus eCrCl and eGFR, were unchanged suggesting that 9 days of IT does not impact upon renal function. In addition, this study suggests that short-term IT does not significantly moderate responses to acute exercise for the serum parameters investigated: FLCs, IgG, IgA, IgM and creatinine.

5. Conclusions

In this study of trained cyclists, 9-days of IT increased resting salivary FLCs and amplified decreases in salivary FLCs in response to acute exercise. This is the first study that has examined FLCs in relation to short-term IT and these findings suggest that salivary FLCs may be sensitive to changes in training load. Increases in salivary FLCs likely reflect alterations in oral inflammation during times of heavy training. Serum FLCs were unaffected by IT, signifying that increases in training volume and intensity only influenced FLCs at a local level. The use of polyclonal FLCs in non-clinical populations and as a prognostic marker has emerged over the last 5 years. The present study extends new research exploring FLCs in saliva highlighting their potential utility as a marker of exercise stress, or as an indirect marker of oral health, relevant to both athletes and the wider population. Long-term studies over the training season and FLC assessments in parallel with oral health examinations are required to explore these possibilities.

Conflicts of interest: None

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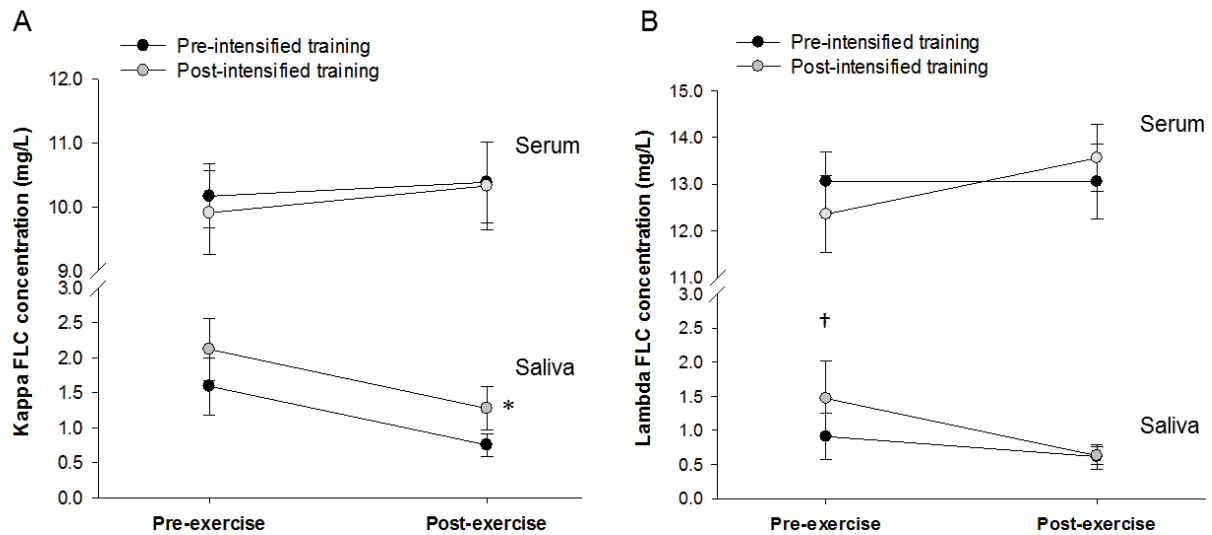


Fig 1 Salivary kappa (A) and lambda (B) free light chain (FLC) concentrations in response to a 1h cycling time trial in trained cyclists before and after a period of intensified training.

A: * Significant decrease in kappa FLC concentration post-exercise compared with pre-exercise, following intensified training only, $p < .05$. B: † Significant elevation in pre-exercise lambda FLC levels post-intensified training compared with pre-intensified training, $p < .05$. Serum FLC levels did not significantly change in response to acute exercise or intensified training

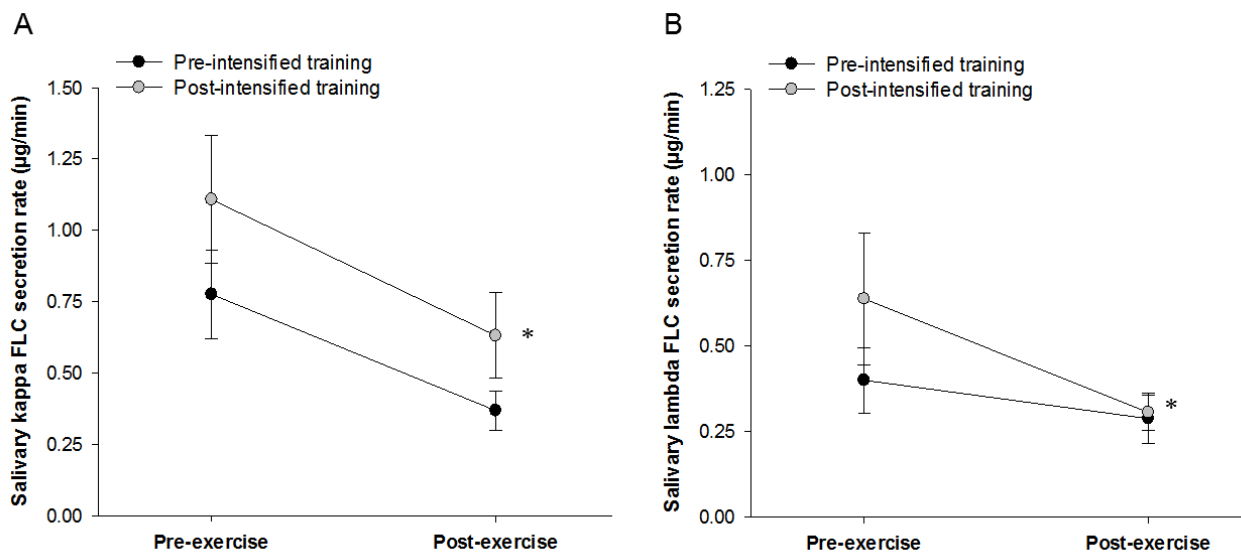


Fig 2 Salivary kappa (A) and lambda (B) free light chain (FLC) secretion rates in response to a 1h cycling time trial in trained cyclists before and after a period of intensified training

A: * Significant decrease in kappa FLC secretion rate post-exercise compared with pre-exercise, following intensified training only, $p < .05$. B: * Significant decrease in lambda FLC secretion rate post-exercise compared with pre-exercise, following intensified training only, $p < .05$

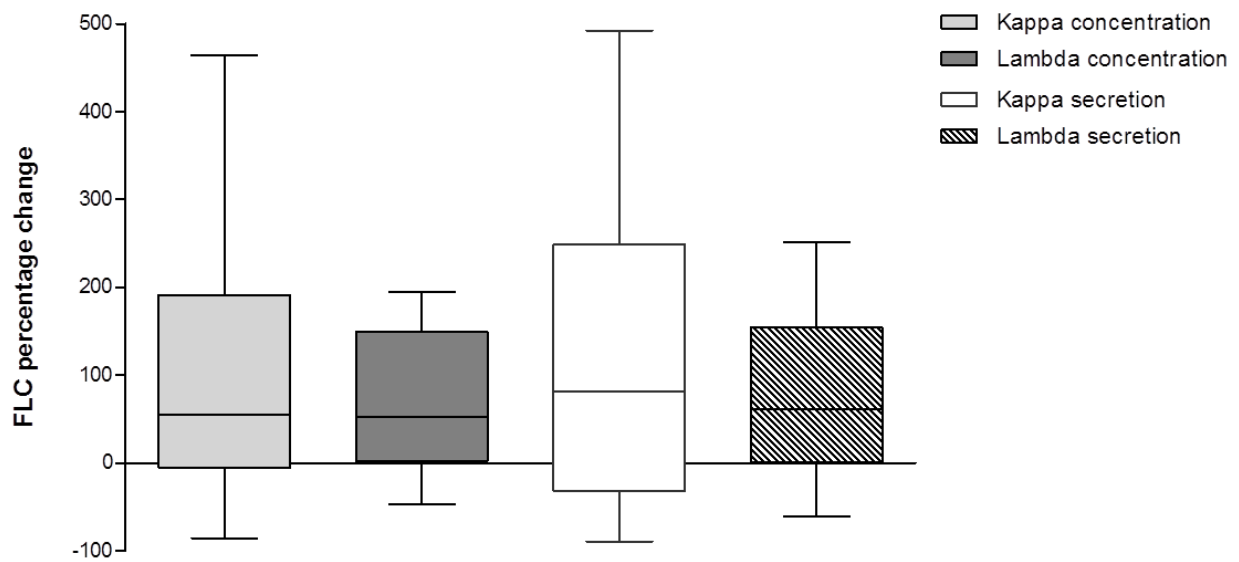


Fig 3. Percentage changes in resting salivary kappa and lambda free light chain (FLC) concentration and secretion rates in response to a period of intensified training. Boxes indicate the 25–75th percentile, the line indicates the median and the whiskers represent the full range.